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A commercial rapid optical immunoassay detects *Streptococcus agalactiae* from aquatic cultures and clinical specimens

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ABSTRACT

The BioStar STREP B Optical ImmunoAssay (STREP B OIA) (BioStar® OIA® Strep B Assay Kit; BioStar Incorporation, Louisville, CO, USA), commonly used for diagnosis of human maternal group B streptococcus (GBS) colonization, was evaluated for its diagnostic and analytical sensitivity and specificity to aquatic animal GBS isolates, cross-reactivity, and diagnosis and recovery of GBS directly from clinically- infected fish swabs. STREP B OIA identified 25 known fish and dolphin GBS isolates. Thirteen non-GBS negative control isolates from fish and other animals were negative, giving 100% analytical specificity and no cross-reactivity. Three groups of 6 Nile tilapia (*Oreochromis niloticus*) (mean weight of 40.60 ± 1.70 g) each were inoculated intraperitoneally with either 10^6 colony-forming units (cfu) GBS/fish, 10^6 cfu *Streptococcus iniae*/fish or 100 μL of tryptic soy broth (TSB) and observed for mortality for 7 days. The nare and brain of all fish were swabbed and subjected to the STREP B OIA for detection of GBS antigen immediately after swabbing (0 h) or 24, 48 and 72 h post-swabbing and compared to conventional culture on trypticase soy agar with 5% sheep blood. The STREP B OIA method demonstrated a diagnostic sensitivity of 75.0% and a diagnostic specificity of 69.2% compared to direct TSA. The percent agreement between OIA and culture was 100%. GBS antigen could be retrieved by OIA following 72-h storage of swabs. These results demonstrate the utility of the STREP B OIA to identify GBS from culture and directly from swabs of clinically- infected fish.

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1. Introduction

Streptococcus agalactiae, group B *Streptococcus* (GBS) causes mastitis in cattle, newborn sepsis and meningitis in humans, and meningo-encephalitis in fish. Recently, Evans et al. (2008, 2009) reported a phylogenetic relationship between human and fish GBS serotype Ia and the infectivity of this human GBS isolate to fish. Given this similarity, it may be possible that mammalian GBS identification techniques could be extended to aquatic species.

Conventional identification of GBS infections, regardless of animal species affected, typically involves microbiological culture and subsequent identification, which usually requires 24–72 h. Commonly used and recommended culture methods include direct plating of swabs onto trypticase soy agar with 5% sheep blood (commonly known as direct TSA) or incubating swabs in selective Lim broth medium before subculture onto blood agar medium (Center for Disease Control, 1996). Rapid immunoassay identification techniques have been employed to detect GBS antigen from human cervical and vaginal swabs. Of the methods reviewed by Yancey et al. (1992), the most accurate non-culture techniques were enzyme immunoassays (EIA).

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Table 1a
GBS isolates[a], USDA-ARS or ATCC designation, fish species of isolate origin, country of origin, and STREP B OIA result.

Designation	Species origin	Country origin	OIA result
01-KU-MU-3B	Mullet (*Liza klunzingeri*)	Kuwait	Positive
01-KU-MU-11B	Mullet (*L. klunzingeri*)	Kuwait	Positive
01-KU-SB-37B	Seabream (*Sparus auratus*)	Kuwait	Positive
01-KU-SB-38B	Seabream (*S. auratus*)	Kuwait	Positive
03-BZ-TN-01	Nile tilapia (*Oreochromis niloticus*)	Brazil	Positive
03-BZ-TN-04	Nile tilapia (*O. niloticus*)	Brazil	Positive
03-BZ-TN-05	Nile tilapia (*O. niloticus*)	Brazil	Positive
03-BZ-TN-06	Nile tilapia (*O. niloticus*)	Brazil	Positive
03-BZ-TN-09	Nile tilapia (*O. niloticus*)	Brazil	Positive
04-AQ-BR-TN-2	Nile tilapia (*O. niloticus*)	Brazil	Positive
04-AQ-BR-TN-3	Nile tilapia (*O. niloticus*)	Brazil	Positive
04-AQ-BR-TN-4	Nile tilapia (*O. niloticus*)	Brazil	Positive
04-AQ-BR-TN-5	Nile tilapia (*O. niloticus*)	Brazil	Positive
04-AQ-BR-TN-6	Nile tilapia (*O. niloticus*)	Brazil	Positive
04-AQ-BR-TN-7	Nile tilapia (*O. niloticus*)	Brazil	Positive
03-IS-ET-09	Hybrid striped bass (*Morone chrysops* × *M. saxatilis*)	Israel	Positive
02-LADL-KI-097	Killifish (*Mugil* sp.)	USA	Positive
05-LADL-TN-108A	Nile tilapia (*O. niloticus*)	Honduras	Positive
97-LADL-KI-151	Killifish (*Mugil* sp.)	USA	Positive
00-LADL-KI-192	Killifish (*Mugil* sp.)	USA	Positive
00-LADL-TN-351A	Nile tilapia (*O. niloticus*)	USA	Positive
90-LADL-ST-503	Hybrid striped bass (*M. chrysops* × *M. saxatilis*) or (*Morone* sp.)	USA	Positive
ATCC 51487	Tilapia (*Oreochromis* sp.)	Israel	Positive
01-KU-Dol-Mus	Bottlenose dolphin (*Tursiops truncatus*)	Kuwait	Positive
ATCC 13813	Bovine (*Bos* sp.)	USA	Positive
ATCC 27956	Bovine (*Bos* sp.)	USA	Positive
M05-61337-3	Bovine (*Bos* sp.)	USA	Positive
M05-61185-10	Bovine (*Bos* sp.)	USA	Positive
M05-61321-3	Bovine (*Bos* sp.)	USA	Positive
M05-63065-3	Bovine (*Bos* sp.)	USA	Positive
510029	Human (*Homo sapiens*)	USA	Positive
510012	Human (*H. sapiens*)	Japan	Positive

[a] Bacterial isolates ($n = 42$) originated from fish, dolphin, bovine, and human sources. Reference isolates were obtained from the American Type Culture Collection (ATCC, Washington DC, USA).

However, sensitivities of these assays compared to culture methods ranged from 20 to 92% or were insufficient to detect low inoculums of GBS. The commercially available BioStar® Optical ImmunoAssay (OIA)® STREP B (Inverness Medical-BioStar Inc., Louisville, CO, USA) was devised to be more sensitive than EIA. This OIA technology allows the observer to visualize the light reflection produced when an antigen–antibody complex forms on a reflective surface. This method is a rapid (20–30 min), inexpensive, and accurate test with 100% analytical sensitivity for inoculums of $>1.4 \times 10^4$ cells/assay (BioStar® Optical ImmunoAssay OIA STREP B package insert, 2006). Such a test would be useful to detect and identify GBS in ante-mortem and post-mortem wild and farmed fish from clinical swabs to expedite GBS detection. The purpose of this study was to evaluate the STREP B OIA for its diagnostic and analytical sensitivity and specificity to aquatic animal GBS isolates, cross-reactivity, and diagnosis and recovery of GBS directly from clinically- infected fish swabs following collection and storage.

2. Materials and methods

2.1. STREP B OIA identification of GBS and non-GBS bacterial isolates

The BioStar diagnostic kit assay, BioStar® Optical ImmunoAssay (OIA)® STREP B (Inverness Medical-BioStar Inc., Louisville, CO, USA) was used to identify known GBS isolates ($n = 32$) of fish, dolphin, bovine, and human origin (Table 1a). Thirteen non-GBS isolates from fish and other animals were also tested as negative controls to determine analytical specificity and cross-reactivity (Table 1b). Since increased bacterial inoculum dose has been shown to have a relationship with increased diagnostic sensitivity of the assay, and since the limits of GBS detection (analytical sensitivity) of the OIA Strep B test is reported to be 1.4×10^4 cells/assay according to manufacturer's instructions, the cell concentration used for each organism in the test procedure was standardized in the following manner. A representative GBS isolate (01-KU-MU-3B) was grown on direct TSA overnight at 30 °C. Sterile cotton tip applicators were used to remove bacterial colonies from the agar plates and bacteria was inoculated in trypticase soy broth (TSB; Remel Corporation, Lenexa, KS, USA[1]) supplemented with 5% sheep blood to the concentration of a McFarland Standard 1 solution (3×10^8 colony-forming units (cfu)/mL). The solution was further diluted 1:856 (to approximately 3.5×10^5 cfu/mL) and 150 μL was sampled to give a final concentration of approximately 5.3×10^4 cfu/assay. To confirm cell density, the diluted sample was further diluted to 1:100 and spiral plated in triplicate on

[1] Mention of trade names or commercial products does not imply endorsement by the U.S. Department of Agriculture.

Table 1b
Non-GBS isolates, designation, species and country of origin and STREP B OIA results.

Designation		Species origin	Country origin	OIA result
S. bovis	ATCC 49133	Bovine (*Bos* sp.)	USA	Negative
S. ictaluri	ATCC BAA 1300	Channel catfish (*Ictalurus punctatus*)	USA	Negative
S. iniae	ARS 60	Hybrid striped bass (*Morone* sp.)	USA	Negative
S. iniae	01-IS-ET-09	Hybrid tilapia (*Oreochromis* sp.)	Israel	Negative
S. iniae	03-IS-ET-02A	Hybrid striped bass (*Morone* sp.)	Israel	Negative
S. iniae	03-IS-DL-12	Hybrid tilapia (*Oreochromis* sp.)	Israel	Negative
S. iniae	03-IS-MO-05	Hybrid tilapia (*Oreochromis* sp.)	Israel	Negative
S. phocae	ATCC 51973	Harbor seal (*Phoca vitulina*)	Norway	Negative
Edwardsiella ictaluri	EILO	Walking catfish (*Clarias batrachus*)	Thailand	Negative
Enterococcus seriolicida (*L. garvieae*)	ATCC 49156	Yellowtail (*Seriola quinqueradiata*)	Japan	Negative
Flavobacterium psychrophilum	07-PS-RBT-02	Rainbow trout (*Oncorhynchus mykiss*)	USA	Negative
Lactococcus garvieae	ATCC 43921	Bovine (*Bos* sp.)	USA	Negative
Staphylococcus aureus	ATCC 33862	Human (*H. sapiens*)	USA	Negative

TSA using an Autoplate 4000 spiral plater (Spiral Biotech, Norwood, MA, USA). Plates were grown overnight at 30 °C and the mean bacterial sample concentration was calculated to 3.5×10^3 cfu/mL with the Q-count plate counter (Model #510; Spiral Biotech). The final dilution concentration of approximately 3×10^5 cfu/mL was confirmed by plate counts, ensuring that bacterial concentrations would be sufficient for STREP B OIA detection.

Thawed frozen (−80 °C) and refrigerated (4 °C) isolates were grown overnight at 30 or 15 °C (*Flavobacterium psychrophilum* only). Pure isolated colonies were later grown overnight at 30 °C on direct TSA or at 15 °C on Shieh agar plates (*F. psychrophilum* only), inoculum prepared as described above in extraction solution and subjected to identification by the STREP B OIA diagnostic kit. Another swab containing bacteria was streaked on direct TSA plate and incubated overnight at 30 °C. For the STREP B OIA tests, cultured and clinical specimens were obtained using swabs provided with the assay kit and samples were processed according to the manufacturer instructions. One positive control swab containing purified strep B STREPococcal antigen and one negative control swab containing wash solution were processed along with bacterial cultures and clinical specimens. The test surface was examined under a bright light source for color changes, which indicated GBS test results. A large solid blue or purple reaction circle surrounding the small solid positive internal control dot (inactivated GBS antigen) was a positive reaction for the presence of GBS antigen (Fig. 1a). A negative reaction occurred when no blue/purple colored reaction circle of any intensity surrounding the internal control dot appeared on the test surface (Fig. 1b). The negative internal control was the unreacted gold test surface surrounding the positive internal control and test surface. A solid blue or purple color over the entire test device surface or a lack of the small solid internal control dot would have been indicative of an invalid result, though this never occurred.

2.2. STREP B OIA identification of clinical isolates post-challenge

Nile tilapia (*Oreochromis niloticus*) with a mean weight of 40.60 ± 1.70 g were housed at the Aquatic Animal Health Research Laboratory in Chestertown, Maryland. Fish were maintained and handled according to Institutional Animal Care and Use Committee (IACUC)-approved guidelines. The fish were kept in 57 L glass aquaria supplied with flow-through de-chlorinated tap water and were maintained on a 12 h:12 h light:dark period. The fish were fed daily to satiation with Aquamax Grower 400 (Brentwood, MO, USA). Daily water temperature averaged 29.00 ± 0.14 °C, mean daily dissolved oxygen was 4.50 ± 0.12 mg/L, and mean total ammonia concentration was 0.16 ± 0.02 mg/L.

Streptococcus iniae (ARS 60) and GBS (01-KU-MU-3B) isolates were taken from frozen samples and cultured overnight on direct TSA at 30 °C. Each pure isolate was diluted in TSB to 1×10^7 cfu/mL, and 100 μL of GBS or *S.*

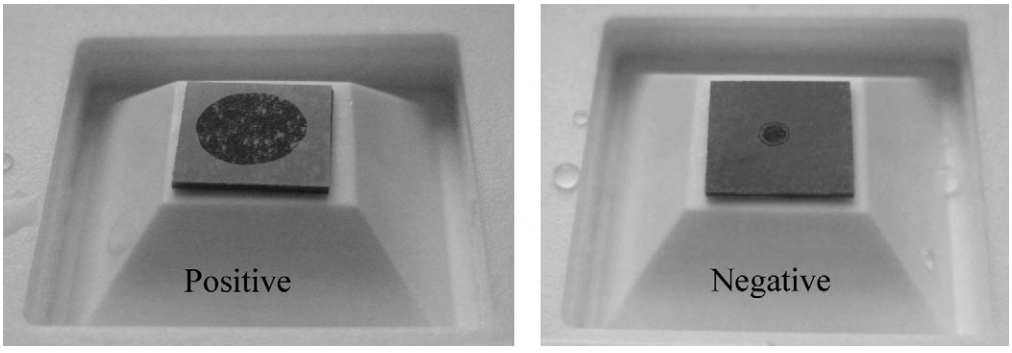

Fig. 1. Positive and negative STREP B OIA GBS reactions when tested with a GBS isolate (ATCC 13813) and an *S. iniae* isolate (ARS60), respectively.

iniae was injected intraperitoneally into 6 fish per group. Six fish were injected intraperitoneally with 100 μL of TSB as a control. Fish were placed into separate tanks according to group, observed for 7 days, and dead fish were removed daily. Fish showing clinical signs or morbidity or fish still alive at the end of the study were euthanized with tricaine methanesulfonate (MS-222; 150 mg/L; Argent Laboratories, Redman, WA, USA). A sterile rayon swab (STREP B OIA) was used to sample nares and brain from all fish, then streaked on a direct TSA plate and cultured overnight at 30 °C. The STREP B OIA was performed as above using the same swab.

In order to assess the viability of the swab samples over time, additional swabs were taken from 2 GBS-challenged moribund fish and held for 24, 48, or 72 h at 22 °C before testing with STREP B OIA and cultured on direct TSA. Each set of swabs was collected sequentially. The identity of cultured bacteria from one GBS-challenged and one *S. iniae*-challenged fish was confirmed using the BIOLOG MicroLog3™ Microbial Identification System (BIOLOG, Hayward, CA, USA) according to manufacturer instructions. BIOLOG results were compared with Microlog and User databases, and a similarity index (SI) of >0.50 and high probability (P) > 90% were considered an excellent identification. All GBS bacteria cultured on direct TSA were additionally confirmed using a Lancefield serogrouping test kit, Slidex strepto kit for grouping of β-hemolytic streptococci groups A, B, C, D, F, and G (bioMérieux Industry).

Given that in any laboratory test there are two distinct kinds of sensitivity and specificity: analytical and diagnostic, the analytical sensitivity and specificity and the diagnostic sensitivity and specificity as defined by Saah and Hoover (1997) are used in this study. Briefly, "analytical sensitivity" represents the smallest amount of substance in a sample that can be accurately measured by the assay while "analytical specificity" refers to the ability of an assay to measure one particular organism in the sample. In a clinical setting, "diagnostic sensitivity" is defined by the percentage of individuals who have the disease and who have positive results on the assay. Conversely "diagnostic specificity" is defined by the percentage of individuals who do not have the disease and who have negative results on the assay. The diagnostic sensitivity of the STREP B OIA method in comparison to the direct TSA culture method was calculated by dividing the number of OIA-positive and culture-positive results by the total number of confirmed positive results. The specificity of the OIA method was determined by dividing the number of OIA-negative and culture-negative results by the total number of confirmed negative results. The percent agreement between the direct TSA culture and commercial OIA was calculated as the sum of results that were both positive and negative, divided by the total number of samples (Carroll et al., 1996).

3. Results

3.1. STREP B OIA identification of bacterial isolates

All of the 32 GBS isolates (fish (23), dolphin (1), bovine (6), and human (2)) (Table 1a) grown on direct TSA and assayed using the STREP B OIA were positive. The ATCC 51487 isolate, originally identified as *Streptococcus difficilis* (Eldar et al., 1994) and later reclassified as *S. agalactiae* (Vandamme et al., 1997; Kawamura et al., 2005), was also STREP B OIA-positive. All of the other 13 non-GBS isolates were STREP B OIA-negative including 6 species (*Streptococcus ictaluri*, *S. iniae*, *Streptococcus phocae*, *Edwardsiella ictaluri*, *Enterococcus seriolicida* and *F. psychrophilum*) from fish, 2 species (*Lactococcus garvieae* and *Streptococcus bovis*) from cows, and 1 species (*Staphylococcus aureus*) from humans (Table 1b). No invalid results or false positives were obtained in the study.

3.2. STREP B OIA identification of clinical isolates post-challenge

During the challenge experiment, fish in the GBS-challenged and *S. iniae*-challenged groups exhibited behaviors suggestive of streptococcal disease: morbidity, remaining stationary at the bottom of the aquaria, lethargy, unresponsiveness, sideways or serpentine swimming, darkened coloration, and little or no feeding response. Seven days after the fish were challenged or injected with TSB, remaining live fish were humanely euthanized.

Table 2
Group, status, organ sampled and numbers and percent of STREP B OIA and direct TSA culture GBS-positive Nile tilapia (*Oreochromis niloticus*; n = 6 for each isolate group) injected with GBS, *S. iniae*, or trypticase soy broth (TSB).

Group	Fish status	Organ sampled	Number OIA positive/number sampled	Percent OIA postive	Number culture-positive/ number sampled	Percent culture-positive
GBS	Dead	Nares	3/3	100%	3/3	100%
		Brain	3/3	100%	3/3	100%
	Euthanized during study; clinical signs	Nares	1/2	50%	1/2	50%
		Brain	0/2	0%	1/2	50%
	Euthanized at 7 days; no clinical signs	Nares	0/1	0%	0/1	0%
		Brain	0/1	0%	0/1	0%
S. iniae control	Dead	Nares	0/6	0%	0/6	0%
		Brain	0/6	0%	0/6	0%
TSB control	Euthanized at 7 days; no clinical signs	Nares	0/6	0%	0/6	0%
		Brain	0/6	0%	0/6	0%

Table 3
Comparison of the results of the STREP B OIA method versus direct TSA culture from 24 fish brain and nare swabs in this study and diagnostic sensitivity and specificity of optical (OIA) and enzyme (ICON, Quidel) immunoassays compared to conventional culture methods from mammalian studies.

TSA	STREP B OIA						Sensitivity (%)	Specificity (%)	References
	# Positive			# Negative					
	Nare	Brain	Total	Nare	Brain	Total			
# Positive	8	7	15	2	3	5	75.0	69.2	This study
# Negative	2	2	4	4	5	9			
TSA		STREP B OIA					53.2	98.4	Baker (1996)
		STREP B OIA					82.5	91.8	Carroll et al. (1996)
		ICON Group B Strep					20.7	99.9	Baker (1996)
		Quidel Group B Strep Test					16.1	98.9	Baker (1996)
Lim Broth		STREP B OIA					72.0	95.6	Park et al. (1996)
		STREP B OIA					62.4	92.2	Carroll et al. (1996)
		STREP B OIA					36.8–100	98.3	Baker (1996)*
		ICON Group B Strep					39.0	99.8	Park et al. (1996)
		ICON Group B Strep					15.1–46	100	Baker (1996)
		Quidel Group B Strep Test					11.9–36	98.9	Baker (1996)
		TSA					69.0	–	Baker (1996)
		TSA					68.0	100	Park et al. (1996)
		TSA					64.4	100	Carroll et al. (1996)

*Inoculum exceeding 10^5 cfu/mL.

Three of the GBS-challenged fish died within 7 days post-challenge, and all samples from these fish were STREP B OIA-positive and culture-positive (Table 2). Two morbid fish exhibiting clinical disease signs were euthanized within the 7-day observation period. One of these fish was STREP B OIA-positive and culture-positive from nare samples, and 1 was culture-positive from brain samples. Neither of these fish were STREP B OIA-positive from brain samples. The fish that previously tested negative, and had no growth on blood agar was frozen and then re-assayed. After freezing the euthanized fish at −20 °C overnight, samples subsequently taken from the nare became 100% STREP B OIA-positive and culture GBS-positive. Samples subsequently taken from the brain were still 0% STREP B OIA-positive. One fish that survived the 7-day study, did not exhibit clinical signs, and was euthanized. This fish was STREP B OIA-negative and culture-negative from nare and brain samples. Cultures grown on direct TSA yielded Lancefield group B positive, Gram-positive, oxidase-negative, catalase-negative bacteria, and BIOLOG identified the isolates as group B *S. agalactiae* (similarity index = 0.78; probability = 99%). The STREP B OIA method demonstrated a diagnostic sensitivity of 75.0% and a diagnostic specificity of 69.2% when compared with direct TSA culture (Table 3). Re-sampling of the euthanized frozen fish increased the sensitivity to 80% and the specificity to 76.9%. The percent agreement between the OIA and culture methods was 100%.

All of the *S. iniae*-challenged fish died within 7 days post-challenge. All samples taken from these fish were STREP B OIA-negative, GBS culture-negative, and *S. iniae* culture-positive. Cultures yielded Gram-positive, oxidase-negative, catalase-negative bacteria, and BIOLOG identified the isolates as *S. iniae* (SI = 0.77; probability = 100%). No TSB-injected fish exhibited clinical signs of disease or died, and samples taken from these fish were STREP B OIA-negative and culture-negative.

When analyzing the viability of the swab samples over time, swab samples from the nare and brain of 2 dead fish were STREP B OIA-positive and culture-positive at 0 h (Table 2) and 24, 48, and 72 h after sampling (Table 4). One 72-h sample was culture-negative but still STREP B OIA-positive.

4. Discussion

The analytical specificity for the commercial STREP B OIA assay was 100% when tested with known aquatic animal non-GBS isolates. No cross-reactivity was noted from 9 fish-derived streptococcal or lactococcal isolates

Table 4
Swab storage time (h) following initial swab sampling (0 h, Table 3 of 2 dead Nile tilapia (*Oreochromis niloticus*; $n = 2$ for each swab storage time) post-challenge with GBS.[a] Numbers and percent swabs taken from the nare and brain STREP B OIA and culture GBS-positive.

Swab storage time (h)	Organ sampled	Number OIA positive/ number sampled	Percent OIA positive	Number culture-positive/ number sampled	Percent culture-positive
24	Nare	2/2	100%	2/2	100%
	Brain	2/2	100%	2/2	100%
48	Nare	2/2	100%	2/2	100%
	Brain	2/2	100%	2/2	100%
72	Nare	2/2	100%	2/2	100%
	Brain	2/2	100%	1/2	50%

[a] Tilapia were injected intraperitoneally with 100 μL of 1×10^7 cfu GBS/mL. Swab samples were held at 22 °C.

similar in biochemical profiles to GBS or from 2 other Gram-positive and 2 Gram-negative fish pathogens. This information extends the list of 31 non-cross-reactive bacterial organisms found in the BioStar® OIA® STREP B literature to 40 organisms.

GBS culture methods can be sensitive and specific, but culture detection of GBS requires viable organisms and greater than 24 h. In contrast, immunologic detection methods can identify GBS antigen regardless of whether the bacteria are viable and in less time. All 32 GBS isolates grew on direct TSA within 24 h and were identified by biochemical and serological methods. The rapid STREP B OIA method demonstrated a 75.0% diagnostic sensitivity and a 69.2% diagnostic specificity compared to direct TSA culture. The percent agreement between the OIA and direct TSA was 100%. The reported diagnostic sensitivities and specificities between direct TSA, selective Lim broth culture, and STREP B OIA from humans have varied. Likewise, diagnostic sensitivity and specificity between enzyme immunoassays (ICON, Quidel) and the OIA have varied compared to either direct TSA or Lim broth culture. Compared to direct TSA culture, the OIA had diagnostic sensitivities ranging from 53.0 to 82.5% and specificities ranging from 91.0 to 98.4% (Baker, 1996; Carroll et al., 1996) (Table 3). The diagnostic sensitivities of enzyme immunoassays (16.0–20.7%) were well below that of OIA. Compared to the GBS selective Lim broth media, the OIA had diagnostic sensitivities ranging from 36.8 to 72% and also exceeded sensitivities of enzyme immunoassays (11.9–36%) (Baker, 1996; Carroll et al., 1996; Park et al., 1996). These results indicate that the OIA is superior to enzyme immunoassays and is as sensitive as conventional plating methods. Much of the variation in diagnostic sensitivity has been attributed to the inoculum concentrations or analytical sensitivity. Overall, diagnostic sensitivity increased 3-fold in all immunoassays when inoculum exceeded 10^5 cfu/mL and sensitivity rose to 100% for STREP B OIA (Baker, 1996). Despite fish inoculums exceeding this concentration, the diagnostic sensitivity of the STREP B OIA was 75.0%. Although GBS growth on direct TSA from swabs was not quantified, it is likely that the swab inoculum or GBS antigen from fish was below that necessary to give positive OIA results.

Although the STREP B OIA is not intended to differentiate between GBS carriers and individuals infected with GBS, a positive STREP B OIA result is presumptive for the presence of GBS and does not necessarily indicate the presence of viable organisms but rather the presence of GBS antigen. We detected GBS antigen from lethally obtained brain swabs and non-lethally obtained nare swabs taken 1–3 days post-injection from dead and diseased fish injected with 10^6 cfu/fish. Thus STREP B OIA can identify GBS in post-mortem and ante-mortem samples and establish GBS as the responsible etiologic agent in fish mortality events. A negative result is presumptive for the absence of GBS but can occur due to antigen levels falling below test detection limits or to inadequate sample collection. Since GBS was not detected from brain and nare swabs of challenged fish at 7 days post-injection, this may represent clearing of the organisms and absence of GBS antigen from fish tissues.

Current GBS detection techniques, whether biochemical (conventional or multi-test systems such as API Strep 20, Biolog, Vitek), serological (Lancefield grouping), or immunological (latex particle agglutination), require culture of the organisms. The ability to rapidly detect and identify GBS directly from fish clinical specimen swabs instead of from cultures offers numerous advantages. It provides results in less than 30 min, unlike culture, which can require up to 72 h. This enables detection of GBS antigen at disease onset from non-lethally sampled fish when etiological information is most critical for intervention. Fish without disease signs can also be non-lethally monitored for disease development. Direct detection of antigen avoids potential problems associated with loss of viability due to inappropriate or prolonged storage, culture overgrowth with other microflora, or delayed transit to the diagnostic laboratory. Swab samples can be stored and transported dry and unrefrigerated for up to 72 h until STREP B OIA testing (BioStar® Optical ImmunoAssay, 2006). Evans et al. (2002a,b) demonstrated the utility of the rayon-tipped Bacti-Swab™ NPG (Remel Inc., Lenexa, KS) with unbroken media ampules to obtain fish clinical specimens and transport viable GBS for up to 4–10 days. This system coupled with the rapid commercial STREP B OIA procedure may extend the time samples could be processed when epidemics occur in remote locations or where no diagnostic facilities exist. Ostroff and Steaffens (1995) reported swabs stored with or without transport media harbored viable but declining numbers of GBS over time, whereas detection of GBS antigen remained consistent even after prolonged storage at temperatures ranging from −20 to 45 °C. Antigen testing performed 2 weeks after swabs were inoculated produced the same OIA signal intensity as the initial controls. Evans et al. (2004) reported 100% recovery of GBS cultured on direct TSA from brain and nare of naturally infected fish frozen for 9 months. There was 100% agreement between fish organs sampled fresh and those sampled frozen. The authors finding that freezing does not hamper GBS isolation from the nare is possible following low injectable doses of GBS and low levels of infection. In the current study, freezing fish and re-sampling nare enhanced STREP B OIA diagnostic specificity and sensitivity.

5. Conclusion

The application of this mammalian STREP B OIA technique to fish and aquatic animal species in the wild, aquacultural settings, and public display aquaria for GBS determination may be possible. Future expanded studies will explore analytical sensitivity and validation of STREP B OIA in aquatic animals and settings.

Conflict of interest statement

No conflict of interest exists for any of the authors.

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